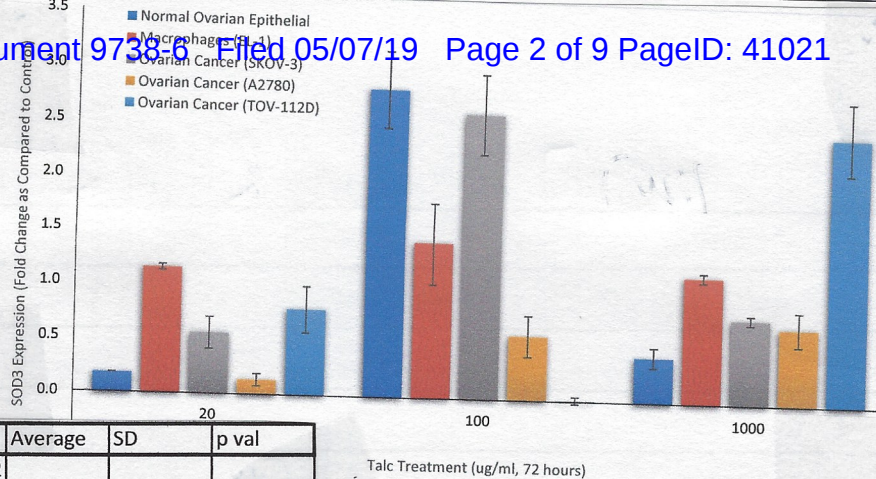


# **EXHIBIT B15**

## **Part 4**





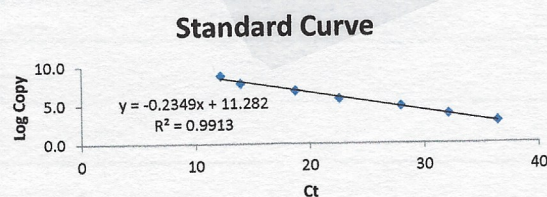
SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.006913	0.010297182			
	0.013535				
	0.010443				
SKOV-3 Control for 100 ug/ml Talc	0.012234	0.011557716			
	0.010882				
	0.050575				
SKOV-3 20 ug/ml	0.019103	0.652865731	0.551015	0.144039	0.05
	0.016749	0.449164129			
	0.022063	0.908963284			
SKOV-3 100 ug/ml	0.038926	2.367972796	2.626045	0.364969	0.05
	0.044892	2.884116792			
	0.026396	1.283859584			
SKOV-3 control for 1000 ug/ml Talc	0.208612	0.1401244			
	0.151128				
	0.129121				
SKOV-3 1000 ug/ml Talc	0.244017	0.741431658	0.770192	0.040674	0.04
	0.23448	0.673367285			
	0.252077	0.798953236			
A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.029232	0.021280566			
	0.028951				
	0.021281				
A2780 Control for 100 ug/ml Talc	0.039562	0.027201344			
	0.026435				
	0.027968				
A2780 20 ug/ml	0.028102	0.320560377	0.133381	0.055191	0.187
	0.024949	0.172406621			
	0.023288	0.09435509			
A2780 100 ug/ml	0.046663	0.715466188	0.584546	0.18515	0.1692
	0.039541	0.453625014			
	0.058702	1.15805571			
A2780 control for 1000 ug/ml Talc	0.052637	0.099050365			
	0.098587				
	0.099513				
A2780 1000 ug/ml Talc	0.178792	0.805058554	0.696194	0.153958	0.1029
	0.157225	0.587328489			
	0.068449	-0.308951611			
TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.030712	0.030110321			
	0.043831				
	0.029509				
TOV112 Control 100 talc	0.016775	0.014654626			
	0.016117				
	0.011072				
TOV112 20 ug/ml Talc	0.03435	1.343995658	0.77735	0.209132	0.1204
	0.028214	0.925229068			
	0.023879	0.629471258			
TOV112 100 ug/ml Talc	0.014484	-0.011613672	0.011113	0.032141	ns
	0.015151	0.033840165			
	0.018271	0.246757232			
TOV112 Control for 1000ug/ml Talc	0.031325	0.028505848			
	0.025687				
	0.068399				
TOV112D 1000 Talc	0.106165	2.724327168	2.490101	0.331245	0.05
	0.47479	15.65588928			
	0.092812	2.255875766			



10/18/2017 Run RT-PCR CAT with standard & samples

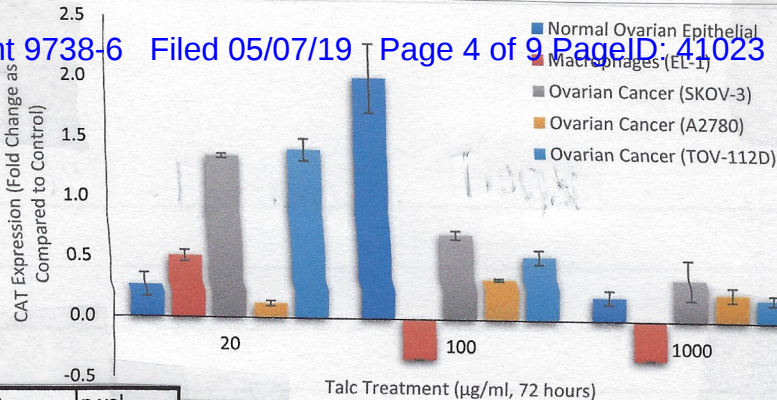
Gene of Interest	CAT	Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	105	bases	
Mass in Daltons	3.21E+04	Da	= number bases x avg. mass/base
Mass in grams	5.32E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.32E-14	ug	= above / 10E-6
Mass in ng	5.32E-11	ng/copy	= above x 10E3

Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.255112	0.277963			
	0.300814				
Normal Ov Epithelial -Control for 200					
500	0.275147	0.196178			
	0.264911				
	0.196178				
Normal Ov Epithelial 20 ug/ml Talc	0.23504	0.198092	0.266425	0.096638	0.161
	0.162371	-0.17233			
	0.261851	0.334759			
Normal Ov Epithelial 100 ug/ml Talc	0.629433	2.208474	2.006022	0.28631	0.05
	0.139599	-0.28841			
	0.55	1.80357			
Normal Ov Epithelial 1000 ug/ml Talc	0.263472	-0.05213	0.197083	0.057972	0.1312
	0.32135	0.15609			
	0.344139	0.238076			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	29.20198	28.39182			
	22.84908				
	27.58165				
EL1 Control DMSO for 100/1000 ug/ml	31.24367	30.90921			
	30.57474				
	33.16323				
EL1 20 ug/ml Talc	25.06856	-0.11705	0.51189	0.047031	0.0765
	41.98112	0.478634			
	43.86952	0.545146			
EL1 100 ug/ml Talc	20.73672	-0.32911	-0.33013	0.001441	0.0189
	17.25388	-0.44179			
	20.67373	-0.33115			
EL1 1000 ug/ml Talc	21	-0.32059	-0.31897	0.002288	0.0189
	21.1	-0.31736			
	22	-0.28824			





SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	2.474985	3.537794			
	3.399213				
	3.676375				
SKOV-3 Control for 100 ug/ml Talc	5.576323	5.393164			
	5.152521				
	5.450649				
SKOV-3 20 ug/ml	9.01118	1.547119	1.34984	0.017963	0.0245
	8.358183	1.362541			
	8.268313	1.337138			
SKOV-3 100 ug/ml	8.554721	0.586215	0.705385	0.035231	0.0462
	9.331777	0.730297			
	9.063065	0.680473			
SKOV-3 control for 1000 ug/ml Talc	14.71117	14.67545			
	14.63973				
	13.97333				
SKOV-3 1000 ug/ml Talc	15.39131	0.048779	0.347002	0.167419	0.2053
	21.50518	0.465385			
	18.03053	0.228619			
A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	6.448358	5.673473			
	5.853449				
	5.493497				
A2780 Control for 100 ug/ml Talc	4.163294	3.876415			
	3.989297				
	3.763532				
A2780 20 ug/ml	7.589043	0.337636	0.121263	0.021465	0.2349
	6.275344	0.106085			
	6.447571	0.136442			
A2780 100 ug/ml	5.154843	0.329797	0.337465	0.010844	0.0691
	5.979143	0.542442			
	5.214293	0.345133			
A2780 control for 1000 ug/ml Talc	9.973024	9.842133			
	11.24123				
	8.312149				
A2780 1000 ug/ml Talc	11.69434	0.188192	0.230282	0.059524	ns
	12.52286	0.272372			
	16.00005	0.625669			
TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	3.382153	3.406415			
	3.150577				
	3.686515				
TOV112 Control 100 talc	0.838564	3.664997			
	3.735731				
	3.594263				
TOV112 20 ug/ml Talc	8.42336	1.472793	1.408291	0.091219	0.0134
	7.983921	1.34379			
	9.243696	1.713614			
TOV112 100 ug/ml Talc	2.319637	0.291634	0.528072	0.058693	0.0419
	2.818786	0.569574			
	2.66972	0.48657			
TOV112 Control for 1000ug/ml Talc	1.807987	1.795893			
	1.783799				
	2.391376				
TOV112D 1000 Talc	2.186972	0.217763	0.189448	0.040043	0.0722
	2.085271	0.161133			
	1.779704	-0.00901			

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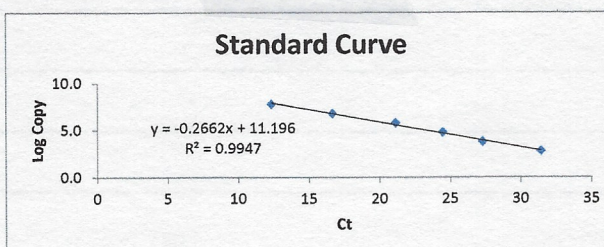


10/18/2017 Run RT-PCR MPO with standard & samples

Gene of Interest	MPO	Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3

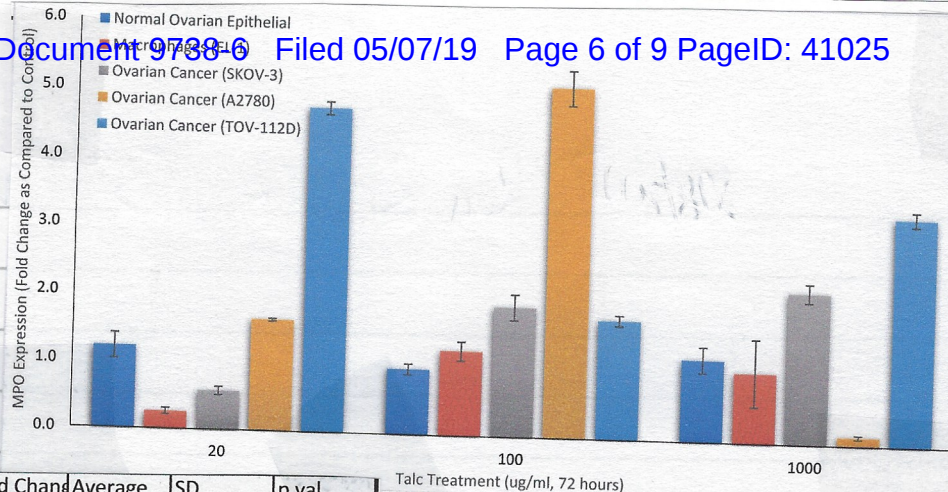
Gene information

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60900000	12.29	7.8
6090000	13.15	6.8
609000	16.12	5.8
60900	20.69	4.8
6090	24.74	3.8
609	28.15	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial -Control for 200 500	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial 20 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
Normal Ov Epithelial 100 ug/ml Talc	0.006142	1.017918	0.962795	0.077956	ns
	0.007321	1.405213			
	0.005807	0.907672			
Normal Ov Epithelial 1000 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	0.026276	0.025624			
	0.024419				
	0.026177				
EL1 20 ug/ml Talc	0.035331	0.37884	0.257395	0.044953	0.0242
	0.033034	0.289182			
	0.031405	0.225609			
EL1 100 ug/ml Talc	0.05	0.951307	1.244003	0.137978	0.0101
	0.055	1.146437			
	0.06	1.341568			
EL-1 1000 control	0.00479	0.004725			
	0.004184				
	0.005202				
EL-1 1000 ug/ml Talc	0.011248	1.380253	1.032749	0.491445	0.1629
	0.003024	-0.36008			
	0.007964	0.685245			





SKOV-3 Cells		fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.022406	0.021915				
	0.021424					
	0.029877					
SKOV-3 Control for 100 ug/ml Talc	0.025145	0.016548				
	0.014775					
	0.018321					
SKOV-3 20 ug/ml	0.027	0.631639	0.571208	0.060431	0.1817	
	0.025	0.510777				
	0.026	0.571208				
SKOV-3 100 ug/ml	0.045764	1.765596	1.897412	0.186416	0.0083	
	0.050127	2.029228				
	0.028656	0.731711				
SKOV-3 control for 1000 ug/ml Talc	0.001542	0.001052				
	0.001059					
	0.001044					
SKOV-3 1000 ug/ml Talc	0.001403	0.333859	2.211632	0.136334	0.008	
	0.003276	2.115229				
	0.003479	2.308034				

A2780 Cells		fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.0108	0.01085				
	0.02					
	0.0109					
A2780 Control for 100 ug/ml Talc	0.063463	0.075407				
	0.072816					
	0.089943					
A2780 20 ug/ml	0.028626	1.638359	1.624942	0.018975	0.05	
	0.026093	1.40486				
	0.028335	1.611525				
A2780 100 ug/ml	0.064579	4.951951	5.130818	0.252956	0.05	
	0.056682	4.224148				
	0.06846	5.309685				
A2780 control for 1000 ug/ml Talc	0.004955	0.004432				
	0.004108					
	0.004233					
A2780 1000 ug/ml Talc	0.004912	0.108294	0.129488	0.029972	0.5279	
	0.005751	0.297523				
	0.0051	0.150681				

TOV112 Cells		fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.088068	0.075135				
	0.070801					
	0.066536					
TOV112 Control 100 talc	0.10722	0.108482				
	0.097003					
	0.121222					
TOV112 20 ug/ml Talc	0.042682	5.583981	4.748687	0.092276	0.05	
	0.036844	4.683438				
	0.03769	4.813937				
TOV112 100 ug/ml Talc	0.019449	2.000104	1.742775	0.075626	0.05	
	0.017434	1.689299				
	0.018127	1.79625				
TOV112 Control for 1000ug/ml Talc	0.008193	0.006483				
	0.005154					
	0.0061					
TOV112D 1000 Talc	0.014068	1.170051	3.334727	0.107619	0.05	
	0.027607	3.258629				
	0.028594	3.410825				



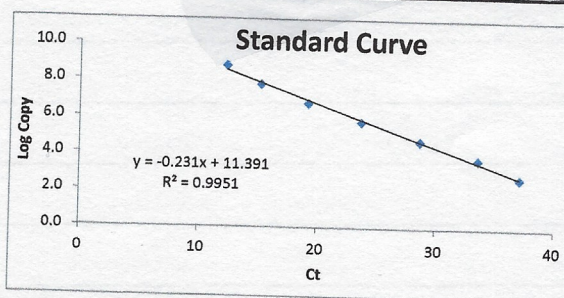
10/19/2017 Run RT-PCR GSTp1 with Standard & samples

Gene of Interest	GSTp1	Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	100	bases	
Mass in Daltons	3.05E+04	Da	= number bases x avg. mass/base
Mass in grams	5.07E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.07E-14	ug	= above / 10E-6
Mass in ng	5.07E-11	ng/copy	= above x 10E3

Gene information

Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8

Standard  
Curve

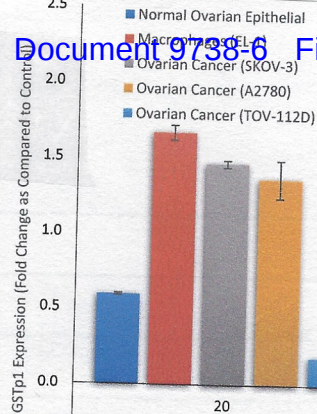


Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial -Control for 200 500	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial 20 ug/ml Talc	7	0.58371	0.592006	0.007273	0.003
	7.05	0.595023			
	7.06	0.597285			
Normal Ov Epithelial 100 ug/ml Talc	6.1	0.38009	0.385747	0.007999	0.004
	6.05	0.368778			
	6.15	0.391403			
Normal Ov Epithelial 1000 ug/ml Talc	6.8	0.538462	0.527149	0.011312	0.05
	6.7	0.515837			
	6.75	0.527149			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	10.20286	10.36977			
	9.75591				
	11.15053				
EL1 Control DMSO (1000 ug/ml volume)	34.79645	33.68753			
	32.57861				
	68.16306				
EL1 20 ug/ml Talc	24.71735	1.383597	1.665897	0.049681	0.0051
	28.00903	1.701027			
	27.28045	1.630768			
EL1 100 ug/ml Talc	19.38792	0.869658	0.959908	0.127633	0.0711
	21.25967	1.050158			
	33.05055	2.187203			
EL1 1000 ug/ml Talc	41.59777	0.234812	0.201666	0.046876	0.0007
	39.36456	0.16852			
	33.1334	-0.01645			

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SKOV-3 Cells					
fg/ul cDNA	Fold Chang	Average	SD	p val	
SKOV control for 20 ug/ml Talc	36.31595	35.95677			
35.5976					
68.59786					
SKOV-3 Control for 100 ug/ml Talc	72.75467	61.72977			
58.02745					
65.4321					
SKOV-3 20 ug/ml	87.92087	1.445182	1.461944	0.023705	0.0116
89.12626	1.478706				
84.90128	1.361204				
SKOV-3 100 ug/ml	71.91819	0.165049	0.09506	0.098979	ns
63.27742	0.025071				
32.76049	-0.46929				
SKOV-3 control for 1000 ug/ml Talc	4.842595	4.837675			
6.244395					
4.832755					
SKOV-3 1000 ug/ml Talc	5.783046	0.195418	0.249358	0.076282	0.1381
3.650555	-0.24539				
6.304928	0.303297				
A2780 Cells					
fg/ul cDNA	Fold Chang	Average	SD	p val	
A2780 control for 20 ug/ml Talc	59.49055	30.29235			
34.42553					
26.15917					
A2780 Control for 100 ug/ml Talc	12.54541	13.77486			
13.56743					
13.9823					
A2780 20 ug/ml	31.33308	1.274657	1.362953	0.12487	0.0342
33.76562	1.451249				
25.47339	0.849267				
A2780 100 ug/ml	9.139274	1.941376	1.983008	0.058877	0.05
9.397987	2.02464				
7.876923	1.535102				
A2780 control for 1000 ug/ml Talc	2.663943	3.107142			
3.176421					
3.481062					
A2780 1000 ug/ml Talc	4.842595	0.558537	0.556953	0.002239	0.0661
6.244395	1.009691				
4.832755	0.55537				
TOV112 Cells					
fg/ul cDNA	Fold Chang	Average	SD	p val	
TOV112 Control for 20 ug/ml Talc	20.39414	20.7106			
16.15828					
21.02706					
TOV112 Control 100 talc	20.17126	19.40386			
22.03957					
18.63647					
TOV112 20 ug/ml Talc	27.94732	0.349421	0.186779	0.037984	0.0394
24.02265	0.15992				
25.13518	0.213638				
TOV112 100 ug/ml Talc	21.15492	0.090243	0.068337	0.030979	ns
16.15982	-0.16719				
20.30481	0.046431				
TOV112 Control for 1000ug/ml Talc	5.996679	6.766235			
7.535791					
9.979309					
TOV112D 1000 Talc	10.08078	0.489866	0.234578	0.089358	ns
8.780969	0.297763				
7.925914	0.171392				

Talc Treatment (ug/ml, 72 hours)





School of Medicine

## Talcum Powder Enhances Oxidative Stress in Ovarian Cancer Cells

Nicole M. Fletcher, Ph.D., Ira Memaj, B.S., and Ghassan M. Saed, Ph.D.  
Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA

### BACKGROUND

- We have previously characterized epithelial ovarian cancer (EOC) cells to manifest a persistent pro-oxidant state as evident by the upregulation of certain key oxidant and downregulation of key antioxidant enzymes.
- This redox state is further enhanced in chemoresistant EOC cells.
- Several studies have suggested a possible association between genital use of talcum powder and risk of EOC; however, the biologic basis for this association has yet to be delineated.

### OBJECTIVE

To determine the effects of talcum powder on the expression of key oxidant and antioxidant enzymes in EOC cells.

### METHODS

- **Cell Culture:** Human ovarian cancer cell lines, SKOV-3 (HTB-77) and TOV-112D (CRL-11731), as well as human macrophages (EL-1, CRL-9854) were all obtained from American Type Culture Collection (ATCC). The ovarian cancer cell line A2780 was obtained from Sigma Aldrich. Human primary ovarian surface epithelium cells from Cell Biologics. Cells were seeded in 60mm<sup>2</sup> culture dishes (1.0 x 10<sup>6</sup>) and allowed to rest for 24 hours.
- **Cell Treatment:** Talcum powder was obtained from Sigma Aldrich and was prepared in DMSO. Cell lines were treated with talcum powder (0, 20, 100, 1000 µg/ml) for 72 hours. Additionally, talc was soaked in DMSO for 72 hours, spun down, and supernatant collected and was used to treat cells (1000 µg/ml, referred to as "supernatant").
- **Real-time RT-PCR Analysis:** Total RNA was isolated from cells utilizing a RNeasy Extraction Kit (Qiagen). cDNA synthesis was performed using the Superscript VILO Master Mix Kit (Life Technologies). Quantitative real-time RT-PCR was performed using a Quantitect SYBR Green RT-PCR kit (Qiagen) and a Cepheid 1.2i Detection System. A standard with a known concentration was designed specifically for β-actin, MPO, NOS, CAT, SOD3, GSR, GPX, GSTp1 using the Beacon Designer software. This allowed for absolute quantification of gene expression as copy numbers per microgram of RNA. Following real-time RT-PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product as a single peak. All samples were normalized to β-actin. A control, which contained all the reaction components except for the template, was included in all experiments.
- **Statistical Analysis:** Data were analyzed using SPSS 23.0 for Windows. Data was analyzed with one way ANOVA followed by Tukey's post hoc tests with Bonferroni correction.

### RESULTS

There was a marked increase in mRNA levels of the pro-oxidant enzymes, NOS and MPO in talc treated ovarian cancer cell line, macrophages, and normal ovarian epithelial cells, all as compared to their control (Figure 1A&B). Additionally, there was a marked increase in the mRNA levels of the antioxidant enzymes CAT, SOD3, GSR, GPX1 and GSTp1, in talc treated ovarian cancer cell lines and in normal ovarian epithelial cells, all compared to their control (Figures 1&2). Interestingly, macrophages had decreased CAT mRNA levels at the 100, 1000, and supernatant doses (Figure 2D).

### RESULTS

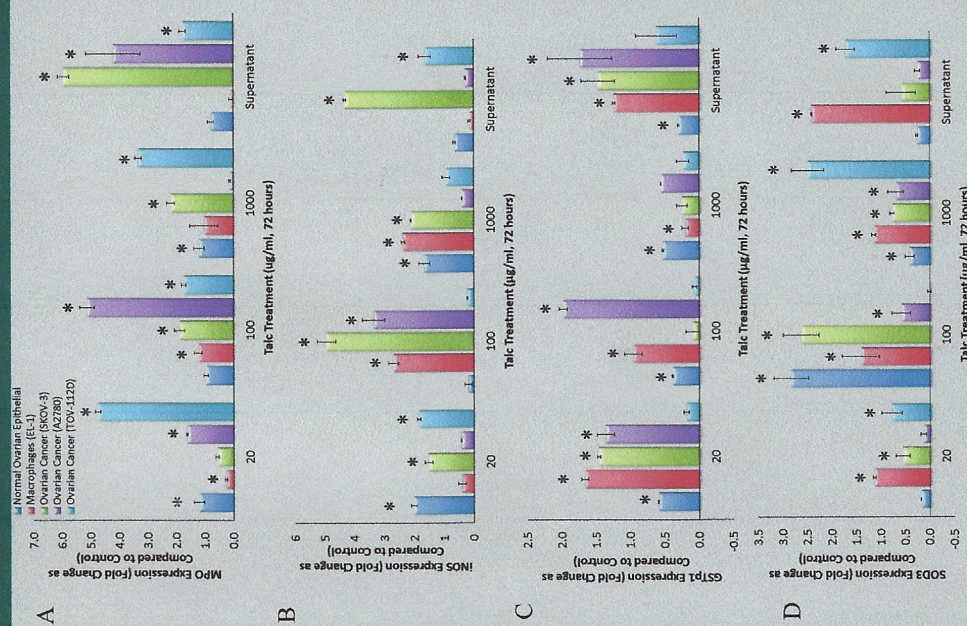


Figure 1: MPO, NOS, GSTp1, and SOD3 Expression in Talc-Treated Cells. Expression of MPO (A), NOS (B), GSTp1 (C), and SOD3 (D) in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3 and TOV-112D) treated with talc (20, 100, 1000) and supernatant (from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. \*P<.05 vs. controls.

### RESULTS

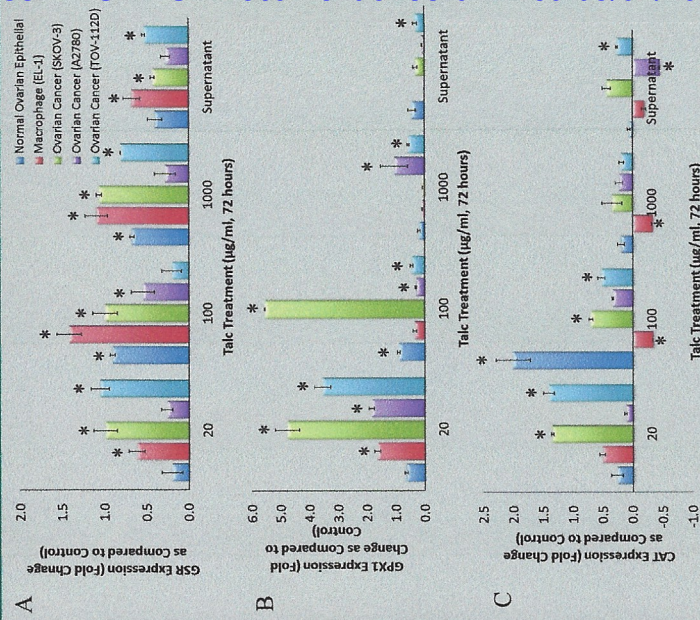


Figure 2: GSR, GPX1, and CAT Expression in Talc-Treated Cells. Expression of GSR (A), GPX1 (B), SOD3 (C) and CAT (D) mRNA levels in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3 and TOV-112D) treated with talc (20, 100, 1000) and supernatant (from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. \*P<.05 vs. controls.

### CONCLUSIONS

This is the first report to show that talcum powder induces a biological effect by further enhancing the redox state in normal macrophages and ovarian epithelial cells as well as in ovarian cancer cells. The results of this study will provide a molecular basis to previous reports that link genital use of talcum powder to increased risk of epithelial ovarian cancer.